Patent
Attorney's Docket No. 000270-007

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Steven L. STICE et al	)	Group Art Unit: 1819
	)	
Serial No.: 08/781,752	)	Examiner: D. Crouch
	)	
Filed: April 1, 1996	)	
	)	
For: CULTURED INNER CELL MASS CELL LINES DERIVED FROM UNGULATE EMBRYOS	)	

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Steven L. Stice, declare and state as follows:

(1) I have been employed by Advanced Cell Technology, as Vice President of Research and Development, since 1994.

(2) I have been an adjunct Professor at the University of Massachusetts since 1994.

(3) Previously, I worked from 1989 to 1994 at American Breeders Service in the area of cloning research.

(4) I have published numerous articles relating to cloning and embryonic stem cells. I am an invited speaker at meetings relating to cloning and embryonic stem cell research. I also

hold numerous patents relating to cloning. My expertise in the art is further established by the attached curriculum vitae.

(5) I have reviewed the Office Action dated 12/30/97 in detail, including the rejection of claims 1-34 and 55-77 under 35 U.S.C. 112, first paragraph. It is my understanding that the Examiner believes that obtaining a live birth by nuclear transfer according to the methods of the present invention would be unpredictable, particularly with the nucleus from a differentiated cell. It is also my understanding that the Examiner believes that the production of chimeric animals lacks reproducibility and predictability. Based on the following results I respectfully disagree.

(6) Please find attached a record of the results recently obtained in our efforts to produce cloned transgenic calves from nuclear transfer between a differentiated donor cell and a bovine oocyte. The nuclear transfers were performed according to the methods disclosed in the present application. In summary, seven live calves have been produced from seven of twelve recipient females. This represents a success rate of over 50%. In particular, I refer to the detailed report which shows that, although six fetuses or calves died in utero or shortly after

birth, the seven surviving calves are, in general, normal and vigorous. In my opinion, these results certainly suggest that live animals may be produced by nuclear transfer from a differentiated donor cell and a bovine oocyte with a high degree of success and predictability.

Furthermore, as detailed in a paper recently submitted to Nature Medicine (copy attached), we have recently had success in producing chimeric calves from both transgenic embryo-derived ES-like cells, and transgenic NT-derived ES-like cells (see pages 6-7 of attached paper). In particular, six calves were born from chimeric embryos that were generated using NT-derived ES-like cells wherein the donor cell nucleus was derived from bovine fibroblasts obtained from a 60-day fetus (see the bottom of page 5). The fibroblasts were transfected with a CMV-β-Galactosidase-neomycin cassette using electroporation before they were used as nuclear donors.

As described on page 6, 8 to 10 ES-like cells were introduced into day 3 *in vitro*-produced embryos, which were cultured *in vitro* until day 7.5 and then transferred into a recipient female. Nine of eleven calves born had multiple tissues that were positive for β-Galactosidase expression (see Figure 3),

including the gonadal tissue. In my opinion, these results suggest that production of chimeric animals can be repeated by one of skill in this art with a high degree of predictability and relatively certain chance of success.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date:_____

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Steven L. Stice